



Heavy metal uptake by plant parts of willow species: A meta-analysis

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ABSTRACT

Previous studies on phytoremediation reported contradictory or inconsistent results on the Cd, Pb, and Zn accumulation in and among plant parts of willow (*Salix*) species. We hypothesized that metals could accumulate in all plant organs in different concentrations and the metal accumulation in tissues would be increased with exposure time. Furthermore, we analysed the effect of soil pH on metal accumulation, and the correlation between metals. We evaluated published information on Cd, Pb, and Zn accumulation in root, stem, twig, and leaf of willow species using meta-analysis. Results showed that all parts of willow species accumulated significantly more Cd, Pb, and Zn in contaminated soils than in uncontaminated soils. However, the metal accumulation was significantly different among plant parts. We concluded that willow species were proven to be prosperous accumulators of Cd (twigs and leaves), Pb (roots and twigs) and Zn (twigs). We found that Cd accumulation rate in stems is higher in soils with lower pH. Significant positive correlation was found between the accumulations of Cd and Zn in stems. Accumulation rates of Cd (both in leaves and twigs) and Zn (in twigs) were increased significantly with exposure time and the accumulation was successful for at least 3 years.

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1. Introduction

Global environmental problems are among the main challenges of the 21st century [1]. Frequent irresponsible and inadequate land use (e.g. managing former and current industrial and mining sites) or demands claimed by population growth (e.g. food supply, urbanization) cause soil pollution, which is one of the most urgent worldwide problems to eliminate [2–5].

Cleaning up contaminated sites with traditional remediation methods places a huge financial strain on stakeholders [6,7]. During the last few decades, researchers found that numerous plant species can accumulate high amount of metal(s) from growing media with low and moderate level of contamination, without suffering severe physiological damages [8–10]. Several studies demonstrated that phytoremediation is an efficient, low-cost, socially acceptable group of remediation processes, establishing long term (years to decades) vegetation cover [11–14]. There are different methods of application of plants in restoring contaminated areas, which depend on the properties of contaminants and the aims of remediation [15]. Besides the phytoextraction mechanism, phytostabilization, phytofiltration, phyto- and rizo-degradation, phytovolatilization and phytohydraulics are also in application to extract, stabilize, filtrate, degrade, volatilize and hydraulically control the extant contaminants or pollutants, respectively [16–18].

In the case of phytoextraction, accumulation rate and availability of metals are functions of various internal and external factors, in-

cluding growth stage, time of exposure, concentrations and forms of metals, interactions between metals, and soil properties [19–21]. Additionally, phytoextraction process can only be successful by sufficient plant density [22]. In the case of plant-heavy metal interaction, two main plant strategy groups can be distinguished: excluders and accumulators [23–25]. The members of excluders concentrate the metal(s) in their roots, while the accumulator plants transfer metal(s) to their aboveground tissues. Metal concentrations are relatively higher in tissues of accumulator plants than in the soil [26–28].

When considering species applicable for phytoextraction, the following features are highly relevant: remarkable metal-tolerance (accumulation potential), high biomass yield and fast growth [29–31]. Among metal-accumulator species, *Salix* (willow) is a widely studied genus [7,18,32]. Based on the results of earlier reports, 10 of the 450 known *Salix* species have been monitored in regard to phytoremediation [33,34]. Besides the common beneficial features of *Salix* species in application for phytoremediation, previous studies reported contradictory or inconsistent results on the rate of metal (in this case Cd, Pb, and Zn) accumulation in and among plant parts [6,35,36]. Within an extensive pot experiment, Dos Santos Utmazian and Wenzel [31] studied the Cd and Zn accumulation potential on several European *Salix* clones. They concluded that metal concentrations were generally higher in leaves than in roots. At the end of a 3-month long exposure time study Rosselli et al. [37] measured higher Zn concentration in roots than in leaves. Studying Cd concentration in seven *Salix* clones, at the end of a 6-year experiment Tlustoš et al. [38] found the highest heavy metal concentration in leaves, followed by comparable concentrations in twigs and wood, while the Cd accumulation in roots was significantly lower. The highest Zn concentration was measured in leaves, while twigs, wood and root tissues accumulated Zn in sig-

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nificantly lower concentrations. They found that Pb was accumulated primarily in roots, followed by wood and twig tissues, while the translocation to leaves was negligible. Heavy metal accumulation of four *Salix* clones was studied by Ruttens et al. [39] who found that Pb concentration was higher in leaves than in stem tissues, in the case of three of the four clones.

The aim of our study was to analyse the available published data on Cd, Pb, and Zn accumulations among the selected plant parts (root, stem, twig, and leaf) of willow species growing in contaminated soils, using meta-analysis. In this paper, we studied the accumulations of Cd, Pb, and Zn, because these metals are involved in phytoremediation studies the most frequently. Furthermore, sufficient amount of data were available for the meta-analysis only in the cases of Cd, Pb, and Zn. We hypothesized that metals would be accumulated in all plant organs, but in different concentrations. In addition, we studied the effect of soil pH on metal accumulation. We studied whether acidic soil pH increased the mobility, availability and accumulation of metals in willows. Furthermore, correlations between the studied metals were also assessed; synergism was hypothesized between Cd and Zn accumulations. Moreover, we investigated if Cd, Pb, and Zn accumulation rates in tissues would be increased with exposure time.

2. Experimental

2.1. Literature search and data selection

We collected data by performing a literature search on Web of Science for the period 1975–2016, using the following search terms: TOPIC = (*Salix*) AND TOPIC = (metal OR phytoremediation). Additionally, we scanned the reference section of the papers found in this search for additional, undetected, relevant publications. To be included, a publication had to report metal (Cd, Pb, and Zn) concentration (\pm SD and sample size) in plant parts (root, stem, twig, leaf) of willow species growing in contaminated vs. uncontaminated (control) soils. Data on tissues referred to as *stem* + *wood* in the papers were classified as *stem* in the meta-analysis. Data were extracted from text, tables, and graphs. Studies in which any compounds (e.g. EDTA) had been applied to facilitate metal uptake were excluded from the analyses, in order to study the inherent phytoextraction capacity of willow species exclusively.

2.2. Statistical analyses

For each uncontaminated-to-contaminated comparison, the unbiased standardized mean difference (Hedges' g) as a common effect size was calculated between uncontaminated and contaminated group:

$$g = J \frac{\overline{X}_U - \overline{X}_C}{S_{within}} \quad (1)$$

$$S_{within} = \sqrt{\frac{(n_U - 1) S_U^2 + (n_C - 1) S_C^2}{n_U + n_C - 2}} \quad (2)$$

and

$$J = 1 - \frac{3}{4(n_U + n_C - 2) - 1} \quad (3)$$

where \overline{X}_U and \overline{X}_C are the mean metal concentration (mg kg^{-1} , dry matter) in parts of willow species growing in uncontaminated (U) and contaminated (C) soils, n_U and n_C are the sample sizes of willow parts from uncontaminated (U) and contaminated (C) soils, and S_U and S_C are their standard deviations. A negative g value means higher metal concentration in willow parts growing in contaminated than in uncontaminated soil. We used subgroup meta-analysis to determine whether metal accumulation from contaminated soils was similar among the different parts of the willow species. The four subgroups were the main parts of a willow species (root, stem, twig, and leaf).

We estimated the overall effect, and examined the effects of moderators (willow parts) using random-effects model. Random-effects model was used, because studies were not expected to estimate a common effect size due to variable locations, conditions, experimental setups and methods used in the individual studies [40]. Random-effect models are more plausible than fixed-effect ones and also attribute the distribution of effect sizes to real differences among studies and do not assume sampling error as the only source of differences in effect sizes between studies [40]. The mean effect size was considered statistically significant if the 95% bootstrap confidence interval (CI; calculated with 999 iterations) did not include zero.

We assessed whether effect sizes were homogenous or varied across studies (i.e., if there was heterogeneity), since if the effect sizes vary across studies, the interpretation of results would be substantially different than in the case of consistent effect sizes. To describe the heterogeneity of effects between studies, complementary measures of heterogeneity, Q , T^2 and I^2 , were calculated [40]. Using a Q -test based on analysis of variance, we partitioned the total variance (Q_{total}) into within groups (Q_{within}) and between groups ($Q_{between}$) variances. Then, these different components of variance were tested for statistical significance [40]. Significant variance between groups ($Q_{between}$) means that metal accumulation from contaminated soils was significantly different according to the plant parts of willow species. Furthermore, to evaluate the proportion of true variance explained by the covariates (subgroup classification), the R^2 was calculated [40]. During the calculations, subgroups with less than six cases were excluded from subgroup analyses.

Meta-analyses are often subjected to publication bias resulting in missing studies and potentially biased effect sizes [40]. Therefore, we tested the publication bias by using funnel plots and the Egger's test [40]. In the case of significant asymmetry, the trim and fill method was used [41]. This method calculates the number of missing studies and estimates their effect sizes as well as standard errors. Then, these missing studies are added to the data set of the meta-analysis, and the summary effect size is re-computed. This method yields an unbiased estimate of the summary effect size [40]. Meta-analyses, heterogeneity measures, and assessment of publication bias were completed by the *MAd* and *metafor* packages [42,43] operated in the R version 3.2.4. To assess whether metal accumulation rate in willow parts increased with exposure time, the relationship between the standardized mean difference (Hedges' g) and the exposure time was examined. We also analysed the correlation between the soil pH and the accumulation rate of metals. Moreover, to study synergism between metals, the correlation between accumulation rate of metals in a given

plant part was assessed. The above relationships were investigated by linear models using the *lm* method implemented in the R.

3. Results

3.1. Literature search

The literature search yielded 527 publications. Of these, only 8 publications reported mean metal (Cd, Pb, and Zn) concentrations with standard deviations and sample sizes for contaminated and uncontaminated (control) soils (Supplementary Materials Table A.1). From these publications, 194 comparisons were extracted. In these comparisons, metal concentrations were found to be moderate to high in soils ($2.5\text{--}67.4\text{ mg kg}^{-1}$ for Cd, $80\text{--}2230\text{ mg kg}^{-1}$ for Pb, and $180\text{--}3718\text{ mg kg}^{-1}$ for Zn). Nine willow species and their clones were studied in the selected papers (Supplementary Materials Table A.1). In the selected publications *Salix viminalis* was studied the most frequently. Trends in metal accumulation rate in plant parts of *Salix viminalis* were similar to those of all combined willow species (Supplementary Materials Fig. D.1), confirming our decision to analyse all willow species together. Only the effect of soil pH on the metal accumulation rate could be analysed because data on other soil properties were missing (there were not data with more than five cases).

3.2. Accumulations of metals in willow tissues

3.2.1. Cd accumulation

All plant parts (root, stem, twig, and leaf) of willow species in contaminated soils accumulated significantly more Cd compared to those of control individuals growing in uncontaminated soils (Fig. 1 and Supplementary Materials Table B.1-2). Accumulation of Cd did not differ significantly among the plant tissues (root, stem, twig, and leaf; Fig. 1 and Supplementary Materials Table B.1-2). Total heterogeneity in the overall model was significant and there was also significant residual, unexplained heterogeneity (Supplementary Materials Table B.1-2). Both the classical and the random-effects version of the Egger's test revealed significant asymmetry in the funnel plot, however, the trim and fill method estimated 0 missing value (Supplementary Materials C.1).

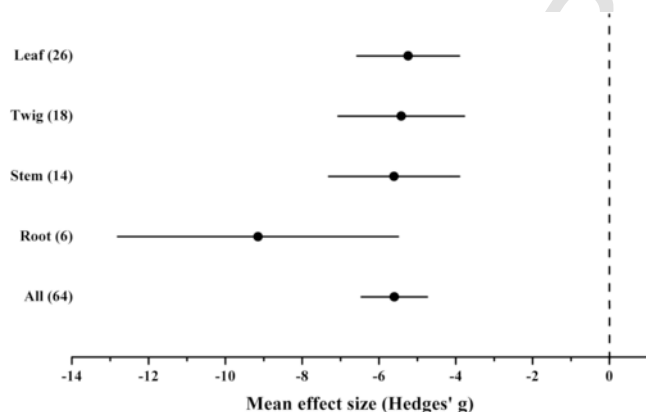


Fig. 1. Mean effect sizes of random-effect models (mean Hedges' $g \pm 95\%$ confidence interval) for Cd accumulation in willow parts. Values in brackets refer to the number of comparisons from which the mean effect size was calculated. A negative g value means higher Cd concentration in plant parts of willow species growing on contaminated than uncontaminated sites. The mean effect size was considered statistically significant if the 95% bootstrap confidence interval (CI) did not include zero.

3.2.2. Pb accumulation

All plant parts (root, stem, twig, and leaf) of willow species in contaminated soils accumulated significantly more Pb compared to those of control individuals growing in uncontaminated soils (Fig. 2 and Supplementary Materials Table B.3-4). However, significant difference was found among Pb accumulation of plant parts, since the accumulation rate of Pb was significantly higher in roots and twigs than in stems and leaves (Fig. 2 and Supplementary Materials Table B.3-4). Both the total and the unexplained heterogeneities were significant (Supplementary Materials Table B.3-4). Both regression tests showed significant funnel plot asymmetries, however, the trim and fill method estimated 0 missing value (Supplementary Materials C.2).

3.2.3. Zn accumulation

All plant parts (root, stem, twig, and leaf) of willow species in contaminated soils accumulated significantly more Zn compared to those of control individuals growing in uncontaminated soils (Fig. 3 and Supplementary Materials Table B.5-6). The accumulation rate of Zn was significantly different among plant parts, as willow stems ac-

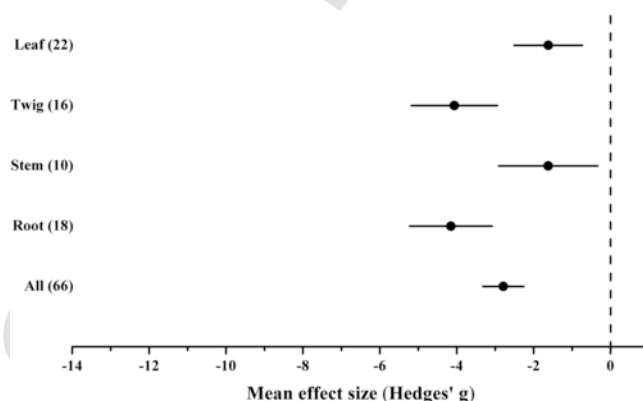


Fig. 2. Mean effect sizes of random-effect models (mean Hedges' $g \pm 95\%$ confidence interval) for Pb accumulation in willow parts. Values in brackets refer to the number of comparisons from which the mean effect size was calculated. A negative g value means higher Pb concentration in plant parts of willow species growing on contaminated than uncontaminated sites. The mean effect size was considered statistically significant if the 95% bootstrap confidence interval (CI) did not include zero.

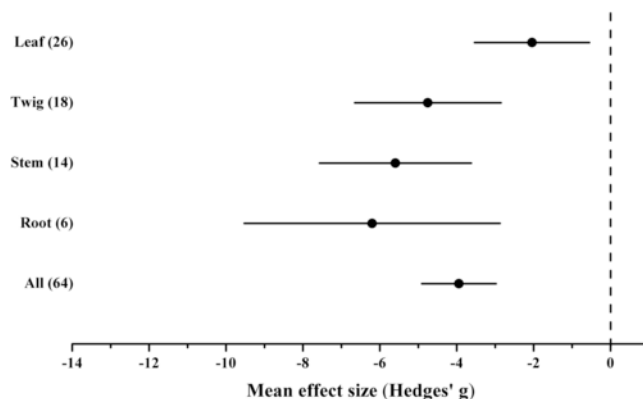


Fig. 3. Mean effect sizes of random-effect models (mean Hedges' $g \pm 95\%$ confidence interval) for Zn accumulation in willow parts. Values in brackets refer to the number of comparisons from which the mean effect size was calculated. A negative g value means higher Zn concentration in plant parts of willow species growing on contaminated than uncontaminated sites. The mean effect size was considered statistically significant if the 95% bootstrap confidence interval (CI) did not include zero.

accumulated significantly more Zn compared to leaves (Fig. 3 and Supplementary Materials Table B.5-6). Both the total heterogeneity and the residual, unexplained heterogeneities were significant (Supplementary Materials Table B.5-6). Both regression tests showed significant funnel plot asymmetries, however, the trim and fill method estimated 0 missing value (Supplementary Materials C.3).

3.3. Correlation between soil pH and metal accumulations in willow tissues

Out of the 12 relationships between the soil pH and the metal (Cd, Pb, and Zn) accumulations in willow parts (in root, stem, twig, and leaf), only one relationship was significant. Significant positive relationship was found between the soil pH and the standardized mean difference (Hedges' g) calculated for Cd concentrations in stems of willow species growing on contaminated vs. uncontaminated sites (Supplementary Materials Fig. D.2), indicating that the accumulation rate of Cd in stems was higher as the soil pH decreased.

3.4. Correlation between accumulations of metals in willow tissues

There were sufficient data only for the concentrations of Cd and Zn in stems and leaves of willow species. Significant positive relationship between the standardized mean differences (Hedges' g) calculated for Cd and Zn concentrations in stems of willow species growing on contaminated vs. uncontaminated sites was found (Supplementary Materials Fig. D.3).

3.5. Temporal accumulations of metals in willow tissues

Analysing the accumulations of Cd, Pb, and Zn in willow tissues, three significant time-dependent correlations were found. Accumulation rate of Cd was increased significantly with exposure time both in twigs and leaves, since the difference in Cd concentrations of twigs and leaves between willow species growing in contaminated vs. uncontaminated soils were increased significantly with exposure time (Figs. 4 and 5). Similarly, the difference in Zn concentration between

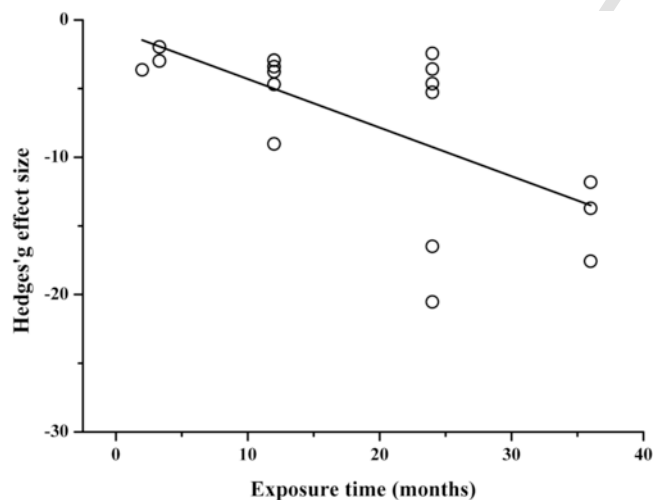


Fig. 4. The relationship between the standardized mean difference (Hedges' g) calculated for Cd concentrations in twigs of willow species growing on contaminated vs. uncontaminated sites and the exposure time. More negative g values indicate higher difference in Cd concentration between the twigs of willow species growing on contaminated vs. uncontaminated sites (linear regression: $F = 11.98$, $n = 18$, $p = 0.003$, $r = 0.39$).

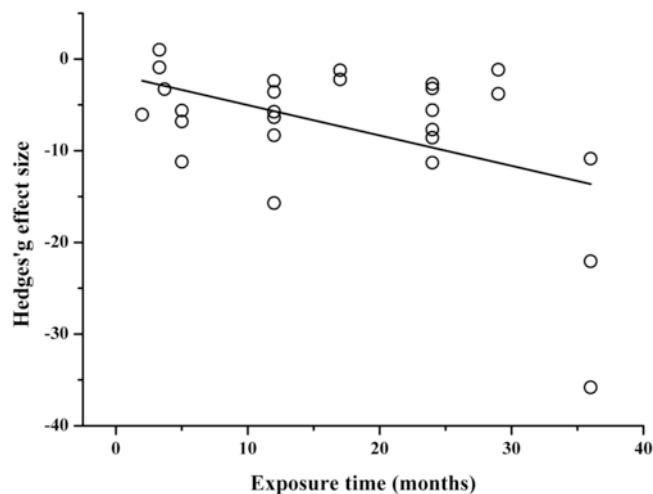


Fig. 5. The relationship between the standardized mean difference (Hedges' g) calculated for Cd concentrations in leaves of willow species growing on contaminated vs. uncontaminated sites and the exposure time. More negative g values indicate higher difference in Cd concentration between the leaves of willow species growing on contaminated vs. uncontaminated sites (linear regression: $F = 6.98$, $n = 26$, $p = 0.014$, $r = 0.19$).

the twigs of willow species growing in contaminated vs. uncontaminated soils was increased significantly with exposure time, indicating that Zn accumulation in twigs tends to increase with time (Fig. 6).

4. Discussion

4.1. Accumulations of metals in willow tissues

Our results based on meta-analytical methods showed that all plant parts of willow species growing in contaminated soils accumulated significantly more Cd, Pb, and Zn compared to those of control individuals growing in uncontaminated soils. However, in some cases, the rate of metal accumulation was significantly different among plant parts.

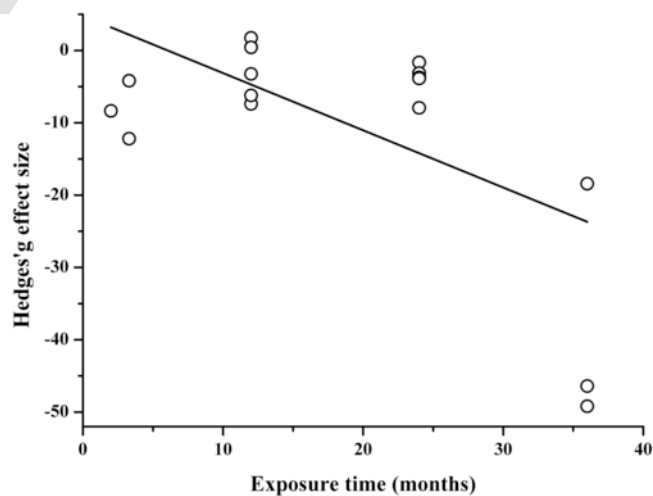


Fig. 6. The relationship between the standardized mean difference (Hedges' g) calculated for Zn concentrations in twigs of willow species growing on contaminated vs. uncontaminated sites and the exposure time. More negative g values indicate higher difference in Zn concentration between the twigs of willow species growing on contaminated vs. uncontaminated sites (linear regression: $F = 8.98$, $n = 18$, $p = 0.009$, $r = 0.32$).

We found that the accumulation of Cd was not significantly different by plant tissues: roots, stems, twigs, and leaves accumulated Cd in similar concentrations. However, previous studies reported inconsistent results. Wahsha et al. [44] demonstrated that roots of willow species accumulated Cd in the highest concentration, while lower concentration was found in leaves and even lower in stem tissues, but these differences were not tested statistically. In the cases of several willow clones Lux et al. [26] found that Cd concentration was significantly higher in roots than in the aboveground plant parts. Our results also showed that roots accumulated slightly more Cd than the other plant parts. Concentration of Cd was also reported to be higher in roots compared to leaves of willows by both low [45] and moderate [14] soil Cd concentrations. Vandecasteele et al. [46] found that Cd concentrations in leaves and roots were similar in willow species growing in moderately contaminated soils, while leaves accumulated more Cd than roots in species growing in heavily contaminated soils. Furthermore, Bissonnette et al. [13] demonstrated that Cd concentration was the highest in stems; it was moderate in leaves, and was the lowest in roots of *S. viminalis*. Ling et al. [47] studied the accumulation of Cd in plant parts of *S. alba* trees by low (0.5 mg kg^{-1}), moderate (6 mg kg^{-1}) and high (60 mg kg^{-1}) soil Cd concentrations. The authors found that Cd concentration was significantly lower in roots than in leaves of *S. alba* both by low and high soil concentrations. Furthermore, similarly to our findings, significant differences were not indicated between plant parts by moderate soil concentration [48]. It was found in a study by Dos Santos Utmazian and Wenzel [31] that leaves of willows accumulated Cd in significantly higher concentration than roots. Generally, compartmentalization of metals within trees is considered to be greatly variable by species and clones [15]. Factors influencing the accumulation pattern of Cd within (among others willow) plants were also assessed by Salt et al. [49] and Vassilev et al. [50]. They found accumulation patterns similar to those in our results and concluded that Cd distribution and translocation to the aboveground tissues was primarily driven by the transpiration stream. In the aboveground tissues of willows, Cd is usually sequestered in the vacuoles, by which toxic effects of Cd can be greatly minimized [51]. Our results on Cd accumulation emphasized that meta-analysis is a powerful statistical approach to summarise the outcomes of several studies with often inconsistent results.

Our results revealed that the accumulation rate of Pb in willow species was significantly higher in roots and twigs than in stems and leaves. In accordance with our results, Bissonnette et al. [13] also reported that roots accumulated significantly more Pb than leaves and stems. Cloutier-Hurteau et al. [48] and Kacálková et al. [52] also concluded that the main Pb depository part of the studied willow species were the roots, since significantly more Pb was accumulated in roots than in leaves. The same accumulation pattern was observed by Borišev et al. [53] in the cases of four different *Salix* clones by various levels of Pb contamination. Mleczek et al. [21] also demonstrated much lower Pb concentrations in the aboveground plant parts compared to roots of *S. viminalis*. Additionally, our results also demonstrated that willow twig is the other main Pb accumulator plant part, as roots and twigs accumulated Pb in similar concentrations. This difference in accumulation rate among willow tissues was most probably caused by the restricted translocation of toxic metals between roots and stems, and between twigs and leaves [54,55]. Restricted translocation of Pb from roots to stems and leaves can be explained by a metal exclusion mechanism in roots by which plants avoid damages in their photosynthetic processes [23,53,56]. Included publications did not assess the role of mycorrhizal associations on metal accumulation; however, it was highlighted that mycorrhizal associations can greatly reduce metal translocation from roots to stems,

among others by binding metals on cell wall components or intracellular immobilization [57,58]. Vysloužilová et al. [59] studied the Pb accumulation in seven different willow clones both by low (29.3 mg kg^{-1}) and high (2029 mg kg^{-1}) soil Pb concentrations. In contrast to our results, the authors found higher Pb concentration in leaves than in twigs of six willow clones by low soil Pb concentration. However, by high soil Pb contamination, concentration was higher in leaves than in twigs in three clones, while higher concentration was demonstrated in twigs than in leaves also in three clones [59]. Accumulation pattern of Pb was also studied by Stanisławska-Głubiak et al. [60], and similarly to our findings, higher Pb concentrations were found in roots than in twigs in the cases of sand and loess soils. However, in contrast to our results, the highest Pb concentration was found in leaves in loess soil. Besides these previous, concentration and translocation patterns of Pb are also widely studied within willows in hydroponics. These results generally emphasize that willows sequester most of the Pb in roots [3,61,62].

We demonstrated that the rate of Zn accumulation was different among plant tissues, as willow stems accumulated significantly more Zn compared to leaves. Contrary to our findings, Maxted et al. [63] and Migeon et al. [64] indicated that the studied willow species accumulated significantly more Zn in leaves than in stems. Moreover, Hammer and Keller [65] and Kacálková et al. [66] showed that leaves can accumulate significantly more Zn than twigs and roots of willow species. Rózanowski et al. [67] also demonstrated higher Zn concentration in leaves than in stems of *S. viminalis*. Yang et al. [33] investigated the accumulation of Zn in plant parts of 12 different willow clones, and they demonstrated Zn concentration in plant parts in decreasing concentration in the following order: leaves > roots > stems. Łukaszewicz et al. [68] also found in *S. viminalis* that leaves accumulated Zn in the highest concentration, however, concentration of Zn was slightly higher directly above the roots than in upper stems. In contrast, Baltrėnas and Čepanko [9] demonstrated that Zn concentration was slightly lower in the middle parts than in the bottom parts of willow stems. Besides Pb accumulation, Vysloužilová et al. [59] also studied the Zn accumulation in seven different willow clones both by low (87.1 mg kg^{-1}) and high (2087 mg kg^{-1}) soil Zn concentrations. By low soil Zn concentration, five willow clones accumulated more Zn in their leaves than in twigs, while only two willow clones accumulated more Zn in twigs than in leaves. By high soil Zn concentration, in contrast to our results, each of the seven clones accumulated more Zn in their leaves compared to twigs. Based on our results it can be highlighted that the Zn accumulation potential of stems appeared to be unexpectedly prosperous compared to the other plant parts of willows. One of the factors potentially accountant for the Zn accumulation pattern demonstrated in our study is the seasonal variation of Zn concentration in stem and leaf [69]. In addition, leaf fall and nutrient availability are also factors resulting in lower Zn concentration in leaves compared to stems [15,70]. Furthermore, co-accumulation with synergistic metals (e.g. Cd) could also contribute to the relatively high Zn concentration in willow stems [19].

It could be concluded that all plant parts (root, stem, twig, and leaf) of willow species growing in contaminated soils accumulated Cd, Pb, and Zn in significantly higher concentrations than those growing in uncontaminated soils. This main finding should be considered during cleaning up contaminated soils with phytoremediation. We recommend that during phytoremediation, attention must be paid to the mechanical removal of roots [71], the one of the main metal-accumulating plant parts of willow species. Without the removal of roots, considerable amount of accumulated metals remain in the soils.

The effect of soil amendments (e.g. manure, red mud, EDTA) on metal mobility in soils and on accumulation in plants have been

widely studied [72,73]. Application of compost was reported to enhance metal accumulation in plants by modifying soil properties, and by increasing soil microbial activity [74]. Application of compost was reported to promote the growth and enhance the metal accumulation potential of willows as well [75]. In contrast, Maxted et al. [63] did not observe any enhancement in Cd and Zn accumulations in willows after sewage sludge application. It was reported that lime and composted biosolid application highly increased Cd, Pb, and Zn concentrations, while reduced growth of willows [36]. Our results showed that willows had the inherent potential to accumulate Cd, Pb, and Zn in high concentrations. This accumulation potential can be further enhanced by adding appropriate amendments.

4.2. Correlation between soil pH and accumulations of metals in willow tissues

We demonstrated that the Cd accumulation in willow stems was significantly higher as the soil pH decreased; suggesting that the more acidic pH may increase metal mobilization and accumulation in stems. Soil pH condition is one of the main factors to consider when assessing metal availability [76]. It was reported that metals were more mobile under acidic soil conditions than in neutral and alkaline soils [77]. In accordance with our observations, Wang et al. [78] found that Cd and Zn accumulations in *Thlaspi caerulescens* plants were increased under acidic compared to alkaline soil conditions. Furthermore, McBride [79] demonstrated that alkaline pH reduces Cd availability. On the other hand, McBride [79] also found that alkaline pH reduces Pb availability as well. In contrast to our findings, Kacálková et al. [52] demonstrated that more alkaline pH (increase from 6.1 to 7.4) increased the amount of available Cd in soil. An earlier study from Shuman [80] observed the same phenomenon that was highlighted by Kacálková et al. [52]. Adameczyk-Szabela et al. [81] studied the Zn accumulation in *Valeriana officinalis* plants grown in acidic (pH 3.5 and pH 4.1), and alkaline (pH 10 and pH 13) soils. The authors found that Zn accumulation was decreased in plants grown under alkaline compared to acidic conditions. Additionally, it was reported that accumulations of metals were inhibited in alkaline soils (pH > 6). Plants, however, are able to modify soil pH, thus affecting the availability of metals [82,83].

4.3. Correlation between accumulations of metals in willow tissues

Studying the correlation between metals, significant correlation was only found between the concentrations of Cd and Zn in stems. The interactions between Cd and Zn ions in plants are quite controversial according to relevant studies [24].

Similarly to our results, Dudka et al. [84] also indicated significant positive correlation between the accumulations of Cd and Zn in *Triticum vulgare* plants. Han et al. [19] studied the accumulations of Cd and Zn, and the correlations between these metals in seven *S. caprea* clones grown in soils with high Cd concentration alone, with high Zn concentration alone, and with combined Cd and Zn contamination. In accordance with our observations, the authors found that Cd concentration was increased by 110% in willow stems in the combined (Cd and Zn) treatment compared to Cd concentration in stems in the treatment with Cd alone. In a complementary study with five willow species, enhanced Cd translocation was observed from roots to leaves in the same combined (Cd and Zn) treatment compared to the treatment with Cd alone [85]. Also, similarly to our results, Landberg and Greger [86] indicated that the presence of Cd had positive effect on Zn accumulation in *S. viminalis* roots. It was also found that the accumulation of Cd was not influenced by the presence of Zn

[86]. In contrast to our findings, Vassilev et al. [50] observed that Cd concentration in soil inhibited Zn accumulation in willows. Han et al. [19] also reported that Zn concentration was decreased by 61% in stems of *S. caprea* in the combined (Cd and Zn) treatment compared to stem Zn concentration in the treatment with Zn alone. In a complementary study with five willow species it was observed that the translocation of Zn was inhibited from roots to leaves in the same combined (Cd and Zn) treatment compared to the treatment with Zn alone [85]. Xu et al. [87] also concluded that high Cd concentration of soil inhibited the accumulation of Zn in *Vetiveria zizanioides* tissues. Furthermore, Hart et al. [88] reported that Cd accumulations in two crop plants (*Triticum aestivum* and *Triticum turgidum*) were inhibited by the presence of soil Zn, while Zn accumulations were also inhibited by the presence of soil Cd, indicating a general antagonism between these metals. Earlier studies demonstrated that simultaneous accumulations of Cd and Zn in plants can be increased by adding chelating agents to soils, such as EDTA and DTPA [63,87,89,90]. Our paper aimed at studying inherent metal accumulations of willows; however, the studies on metal accumulations by adding chemicals are also necessary parts of remediation analyses.

4.4. Temporal accumulation of metals in willow tissues

The effect of exposure time (duration of experiment) was quite variable among studies on different plant parts. Considering the total number (12) of potential cases, significant correlation was proven between metal accumulation and exposure time in only three cases.

In our study, increasing accumulation of Cd was shown both in leaves and twigs of willow species exposed to elevated Cd concentration for a longer time. Contrary, Wieshammer et al. [6], studying Cd accumulation in leaves of four willow species in a 3-year long experiment, found that Cd accumulation was decreased from year to year. They concluded that this decreasing trend of accumulation was corresponded to the decreasing amount of available Cd in the soil. However, similarly to our results, in a 3-year long study French et al. [91] also found that Cd concentration was increased in leaves of all the five studied willow species from the second to the third year of the experiment. Experiments studying Cd accumulation in willow twigs are very limited [52,60], in addition none of these studied the temporal trend of accumulation.

In our study, there was no significant relationship between the accumulation rate of Pb in willow tissues and the exposure time. There are only a few studies reporting information on Pb accumulation in willow parts related to exposure time. In a 4-year long study, Zárubová et al. [92] found that concentration of Pb in wood tissues was increased by the second year, was slightly decreased by the third year and was increased again by the fourth year of the experiment. Ruttens et al. [39] also showed variations in the accumulation of Pb in leaves of seven willow species. They found that two species had significantly increasing; two had barely increasing, while three species had decreasing concentrations in tissues from the second to the third growing season. Based on the above, it seems that Pb accumulation in willow parts can be varied greatly with exposure time.

Our results indicated that Zn accumulation in twigs was tended to increase with time, as the difference in Zn concentration between the twigs of willow species growing in contaminated vs. uncontaminated soils was increased significantly with exposure time. Other studies (that were not involved in our meta-analysis) evaluating temporal accumulation rate of Zn in willow twigs are missing. However, Rosselli et al. [37], studying accumulation rate of *S. viminalis*, reported that leaves and stems accumulated significantly more Zn after 8 months of the plantation than did after 3 months. They found, that accumula-

tion rate of Zn in roots was also increased, but not significantly. Similarly, Mleczek et al. [93] found that willow roots accumulated more and more Zn with time. Similar to earlier studies [37,93] our results also suggest that Zn accumulation in willow parts seems to be increased with time.

Despite the relatively fast growth of willow species, it seems that dilution effect (when intensity of biomass production exceeds the intensity of metal accumulation, resulting in decreasing metal concentrations in concerned plant parts) does not occur during their metal accumulation [55], as significantly increased accumulation rate of Cd in leaves and twigs, and Zn in twigs were shown with exposure time. Although, we did not observe any significant correlations in most cases (9 cases) of the metal-plant part relationships, there are studies showing temporal changes of Cd and Zn accumulation in willow tissues [91,94]. In the cases of accumulation rate of Cd in leaves and twigs, and Zn in twigs we could not identify saturation point, indicating that metal accumulations were continuous until the end of the 36-month long period. Accumulation capacity of willow species was also investigated for longer time periods. In a decades-long experiment Riddell-Black [95] concluded that to reduce metal concentrations in a heavily contaminated soil to a target concentration, willows should be left growing in highly contaminated soils over 30 years. Similarly, Ostman [96] pointed out that willow plantations need at least 20 years to decrease soil concentration from high to low, assuming constant extraction rate. However, the decontamination of moderately contaminated soils could be achieved in a reasonable timescale (3–5 years) if willows cultivated in short rotation [15]. In addition, Mertens et al. [69] found that after 4 and 6 years from establishment of willow plantation, leaf and stem tissues had significantly lower Cd and Zn concentrations than after the first and second years of the experiment. As the success of phytoextraction by willow species depends on both the accumulation rate of the plant parts and the accumulation rate over the time period [97], based on our results, we suggest that willow plantation should be left growing in contaminated soils for at least 3 years, especially when cost-efficiency and enhanced metal extraction are of particular importance. Of course, for phytoremediation purposes, willow plantations could be effectively grown for even much more than 3 years, as some willow parts could be practicable depositories for heavy metals even after decades [98].

We showed that all willow parts accumulated the studied metals (Cd, Pb, and Zn) significantly, and metal accumulation in certain harvestable tissues was continuous for 36 months. Although the covariates (classification of willow parts) accounted for a considerable proportion of the true variance, there remained significant unexplained heterogeneity in all models. Moreover, the variance explained by the covariates (classification based on plant parts) was also low, indicating the importance of other factors on metal accumulation. This heterogeneity could arise from differences in study regions, study designs and methods applied in the individual studies. Besides the importance of the different plant parts identified in our study, other inherent features of metal accumulation (e.g. concentrations, forms and available amount of metals in the contaminated soil [20,52], soil type and other soil properties [21,99], interactions between soil properties and metals [100,101], types of willow clones [1,31], growth stage of species [63], plant density [102], and time of exposure [22,69] could also contribute to heterogeneity. Therefore, all of the abovementioned essential factors must be considered in a global meta-analysis on phytoremediation.

5. Conclusions

Based on the results of our meta-analysis we highlight as a novel observation that willows were capable of accumulating Pb in high concentrations also in twigs. Furthermore, besides leaves, stems of willows also appeared to be successful Cd accumulators. Accumulation rates of Cd and Zn were high until 36 months, meaning that willows are successful accumulators of these metals in such a time scale. Significant effect of soil pH on Cd accumulation in stems was also demonstrated, but influence was not found in the cases of other metals and plant parts. This indicated that metal accumulation was universally not dependent on soil pH within the studied soil pH range (pH 5.3–8.2). Significant positive correlation was found between the accumulations of Cd and Zn in willow stems, suggesting that stem is a useful plant part in phytoremediation of soils with simultaneous Cd and Zn contamination. Considering our results we recommend that during phytoremediation attention must be paid to the removal of roots at the end of the remediation process, since without the removal of roots considerable amount of accumulated metals remain in the soils. Furthermore, to exploit the inherent metal accumulation potential of willows, we suggest that willow individuals should be left growing for at least 3 years in the contaminated soils, knowing that metal accumulations are continuous and intensive in such a time scale. Additionally, it is highly required to conduct long term (3–10 years) studies in order to assess long term metal accumulation potential and the time scale of economical applicability of willows more thoroughly. However, the successful growing of willows, and therefore metal accumulation rates, are functions of several factors that should also be addressed in further studies.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.jhazmat.2017.03.068>.

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